Lygus hesperus (Hemiptera: Miridae) Feeding on Cotton: New Methods and Parameters for Analysis of Nonsequential Electrical Penetration Graph Data

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ABSTRACT This study is the first to statistically analyze the stylet probing/penetration behaviors of *Lygus* (Hemiptera: Miridae) bugs, and the external body movements associated with both probing and nonprobing, via electrical penetration graph (EPG) and videorecording, respectively. Behavioral quantification allows powerful statistical comparisons among host plants or other treatments. Thus, statistical analysis of data has played an important role in EPG research. However, few attempts have been made to standardize types and terminology used for statistical parameters. We provide here the first complete system of organization and terminology for nonsequential EPG parameters. Widespread adoption of these terms will allow standardization in EPG research. Our EPG and video data reveal for the first time the stylet penetration behaviors of nymphal *L. hesperus* that cause cotton square damage, and the mechanism involved. *L. hesperus* nymphs spent only 15% of their time on squares probing; the remainder was spent standing motionless in place, grooming, or in sensory exploration. While probing, two thirds of their time was spent in laceration/salivation and one third in ingestion. Thus, *L. hesperus* nymphs actively spread out numerous, minute injections of their macerating watery saliva, deeply drilled/lacerated into all parts of the developing square. After injection of saliva within the square, the insect then stands and waits for solubilization of the square's cell contents, and then quickly ingests the slurry. The extensive laceration by the stylets may, secondarily, potentiate salivary maceration by mechanically rupturing cell walls. The plant responses to such behavior are thus summarized as "mechanical cell rupture-enhanced maceration."

KEY WORDS electronic monitoring of insect feeding, EPG, probing, stylet penetration, plant bug

Our ability to discern hemipteran stylet activity within plant tissue was revolutionized by the method of McLean and Kinsey (1964) that included the insect as part of an electrical circuit, now universally termed electrical penetration graph (EPG) monitoring of insect feeding. Tjallingii (1978), Backus and Bennett (1992), and others improved this technique, and in the 40 yr since its invention (for review, see Backus 1994), nearly 200 papers have been published; most emphasized aphid and leafhopper feeding (Backus 1994, Walker and Backus 2000).

One of the most powerful abilities EPG offers researchers is quantification of complex behaviors occurring inside the plant. Statistical analysis of data has played an important role in EPG research. However, few attempts to standardize statistical methods have been made, especially types and terminology used for statistical parameters. Van Helden and Tjallingii (2000) reduced confusion by reviewing standards for experimental design and statistical parameters. They defined and discussed parameters that are sequential (i.e., making up information inherent in the sequential order of the waveforms within a probe) and nonsequential (i.e., making up information irrespective of waveform order within a probe). However, in the majority of their analyses, Van Helden and Tjallingii (2000) emphasized sequential parameters because of the importance of such parameters for aphid studies. Aphids and other sheath-feeding hemipterans perform stereotypical behavioral sequences during stylet penetration. In contrast, nonsheath-feeding hemipterans (i.e., lacerate-and-flush feeders, including cimicomorphan heteropterans such as *Lygus* spp.) usually are less sequential in their behavior; thus, nonsequential parameters often are more appropriate than sequential.

Lygus species plant bugs are agriculturally serious hemipteran pests that cause yield losses to alfalfa,

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Medicago sativa L. (Sorenson 1936); common bean, *Phaseolus vulgaris* L. (Elmore 1955); cotton, *Gossypium hirsutum* L. (Cassidy and Barber 1939); and other fruit and seed crops (Kelton 1983, Middlekauff and Stevenson 1953, Allen and Goede 1963, Wise and Lamb 1998). On cotton, nymph and adult *Lygus hesperus* (Knight) feed on vegetative and reproductive structures (Mauney and Henneberry 1979, Snodgrass 1998, Wilson et al. 1984). Nymphs prefer developing squares (i.e., immature fruiting structures which develop into mature cotton bolls), whereas adults prefer vegetative structures (Snodgrass 1998). The extent of injury to cotton is described by Leigh et al. (1988), who notes that, before 1 August (in the Northern Hemisphere), an individual *L. hesperus* has the potential to cause abscission of 23,400 squares per ha. Much of this damage is caused by nymphs (Gupta et al. 1980). The severity of nymphal impact on cotton motivated this research, which is the first quantification of *Lygus* feeding on cotton reproductive structures using EPG.

The exact feeding strategy of mirid (cimicomorphan) hemipterans such as *Lygus* spp. is still not clear. They were long considered lacerate-and-flush feeders (Miles 1972, Backus 1988), a strategy that recently was renamed cell rupture feeding (Backus et al. 2005). These insects use stylets to vigorously lacerate plant cells in a small area, simultaneously secreting watery saliva into the ruptured cellular matter, and then ingest the resulting lacerated/macerated "soup." However, mirids were placed in a new strategy, macerate-and-flush, by Miles and Taylor (1994), after those authors observed a South Asian mirid, *Helopeltis clavifer* (Walker), to move its stylets very little while probing, instead apparently relying only on highly enzymatically active saliva to solubilize cell contents. Determining whether *Lygus* spp. are lacerate-and-flush or macerate-and-flush feeders is important, because this information will dictate the precise mechanism of plant damage. In turn, understanding the mechanism will aid in development of transgenic host plant resistance, by suggesting specific traits or gene combinations to select or engineer for resistant plants. EPG is the sole, and most rigorous, tool available to answer this question.

Cline and Backus (2002) presented the first EPG ethogram (including characterization and correlation of waveforms) for any cimicomorphan heteropteran, for third instars of *L. hesperus.* Our goal here is to build on Cline and Backus (2002), initiating a long-term study of *Lygus* stylet penetration and its role in plant damage. Specific objectives include 1) measure, via EPG, and statistically describe stylet penetration behaviors; 2) analyze, via video observation, body movements and plant locations of EPG-monitored nymphs; and 3) use statistical description of these data as an example of a hierarchical scheme for organization, terminology, and analysis of nonsequential EPG parameters. This research could be the first step in developing a stylet penetration index for *L. hesperus* on cotton, similar to that of *Empoasca kraemeri* (Ross & Moore) (Serrano et al. 2000) on *Phaseolus* sp.

Materials and Methods

Insect and Plant Rearing

Detailed methods are available in Cline and Backus (2002). In summary, *L. hesperus* nymphs were reared from eggs (BioTactics, Riverside, CA; APHIS import permit no. 33219) on commercial diet (BioServ Inc., Frenchtown, NJ), under a photoperiod of 16:8 (L:D) h at 27:23°C and 60–70% RH. Cotton ('Coker 312') seed was planted with ProMix and supplemented with Osmocote (14–14-14) fertilizer pellets. Plants were grown in a growth chamber under a photoperiod of 16:8 (L:D) h at 30:34°C. Plants were used 6 wk after planting when multiple squares were visible. Insects were pretest conditioned on cotton plants for 48 h before EPG monitoring.

EPG Monitoring

Nymphs were simultaneously EPG-monitored and videotaped one at a time on cotton squares. Nymphs were collected, attached to a 12.7-μm-diameter gold wire tether (Sigmund Cohn Corp., Mt. Vernon, NY) with silver conducting paint (in *n*-butyl acetate solvent; Ladd Industries, Burlington, VT), as described in Cline and Backus (2002). One wired nymph was acclimated for 1 h on the external surface of a cotton square and then starved on a Plexiglas plate for 1 h. EPG monitoring and videomicrography were simultaneously initiated before placement on the test plant (cotton square). Each insect was recorded for a minimum of 2 h. Third instars were used because of their severe impact on crops (Gupta et al. 1980) and because little is known of their feeding behavior. In addition, body size is similar to *Empoasca* leafhoppers, allowing use of similar monitor settings and wiring protocols.

An alternating current (AC) EPG "Missouri Monitor," type 2.2 (Backus and Bennett 1992) with a fixed input resistor level of 1 MΩ (10^6 Ω) was used. A 75-mV, 500-Hz AC electrical signal was applied to the test plant. Electrical resistance changes during stylet penetration were amplified, rectified, and recorded as changes in voltage by analog-to-digital conversion by using a Gateway Pentium computer with Windaq hardware and software (Dataq Instruments Co., Akron, OH). Insects still probing at 2 h were allowed to continue until completed; thus, no probe was artificially terminated. All recordings were made within a Faraday cage to alleviate external electrical noise. Once recordings were finished, insect and plant were discarded.

Videomicrography

A Javelin (model no. JE3462HR) video camera (Adlon Instrument Co., St. Louis, MO) was mounted on a trinocular Wild M-5 Apo stereomicroscope. The camera/microscope apparatus was connected to a Panasonic AG-6740 (Will Electronics, St. Louis, MO) time-lapse videocassette recorder. Recordings were made on Sony high-resolution videocassettes at a 6-h setting

to maximize the number of frames (eight per second) captured.

Statistical Analysis

Measurement of Waveform/Video Observations. Waveforms were displayed postacquisition by Windaq Waveform Browser software. Durations of waveform events (length of one particular waveform type uninterrupted by another waveform type) were sequentially measured and entered into a modified Excel spreadsheet by using a Windaq notepad and Excel macro program (Van Giessen and Jackson 1998). Video analysis was performed on a frame-by-frame basis (Cline and Backus 2002). Video times and activities were entered into an Excel worksheet, and each activity's duration calculated.

Definitions of Nonsequential Parameters. We present herein, for the first time in totality, a method for identifying and organizing nonsequential parameters including statistical frequencies (i.e., numbers of occurrences) and durations used to analyze EPG data. This method originally was described in Serrano 1997, then some parameters were further expanded in Serrano et al. 2000 and Almeida and Backus 2004. The definitions are intuitive and mathematical, and conceptually they are similar but more complete than similar definitions from previous articles (e.g., Backus 1988, 1994; Backus and Hunter 1989; Calderon and Backus 1992; Van Helden and Tjallingii 2000).

The notation used in the mathematical definitions is explained as follows. Let

E_{hijkl} = A count of the l^{th} event within the k^{th} waveform type within the j^{th} probe within the i^{th} insect within the h^{th} cohort. Each E_{hijkl} has a frequency of 1.

W_{hijk} = A count of the k^{th} waveform type within the j^{th} probe within the i^{th} insect within the h^{th} cohort. Each W_{hijk} has a frequency of 1.

P_{hij} = A count of the j^{th} probe within the i^{th} insect within the h^{th} cohort. Each P_{hij} has a frequency of 1.

N_{hi} = A count of the i^{th} insect within the h^{th} cohort. Each N_{hi} has a frequency of 1.

D_{hijkl} = The duration of the l^{th} event within the k^{th} waveform type within the j^{th} probe within the i^{th} insect within the h^{th} cohort.

where

$h = 1, 2, 3 \ldots a$, the number of cohorts (i.e., treatments)

$i = 1, 2, 3 \ldots n$, the number of insects

$j = 1, 2, 3 \ldots p$, the number of probes

$k = 1, 2, 3 \ldots w$, the number of waveform types

$l = 1, 2, 3 \ldots e$, the number of waveform events

The letter N represents the number of insects used in a treatment (i.e., a cohort) and N_T represents the total number of insects in the experiment. Therefore, N_T is mathematically defined as

$$N_T = \sum_{h=1}^{a}\sum_{i=1}^{n} N_{hi} \qquad [1]$$

Some parameters are calculated for each individual insect so that each insect's data can be compiled before being averaged. A compiled parameter for an insect is termed "by insect," and averaged parameters across N insects are termed "per insect." Parameters calculated by probe and by event can then be averaged per probe and per event in a similar manner. For example, there is a parameter termed number of waveform events by insect (NWEi; equation 6), and when this parameter is averaged for a cohort, it is termed number of waveform events per insect (NWEI; equation 7). To reduce the name length of these terms, we do not include "mean" in each name, i.e., mean number of waveform events per insect. Therefore, any "per"-containing parameter name is considered a mean. In abbreviations, all letters are capital except "by" factors, which are lowercase (see previous example).

When dealing with complex data sets, it sometimes is easier for SAS to arrive at a final mean by taking a mean of a mean of compiled data. In such a case, the final mean is similar and sometimes identical to the mean of a mean, and the two parameters can be interchangeable. For example, waveform duration per probe (WDP) is a final mean, whereas waveform duration per probe (per insect) (WDPI) is a mean of a mean. By convention, the parameter name shows the second mean in parentheses. The portion of the parameter name not in parentheses denotes the heuristic level of the parameter (Fig. 1) (e.g., WDPI resides at the probe level, as does WDP).

The parameters in equations 2 and 3 below are presented mathematically for clarity's sake; they are behaviorally meaningful as indicators of the homogeneity of insects used in this research. However, because these parameters are "total" values that represent a sum for all insects in the cohort (i.e., total number of insects in treatment), they are not statistically testable. Many of the other parameters are means and can be used in statistical analyses. For clarity and easier identification, Table 1 names the parameters and lists abbreviations and equation numbers. Table 2 lists additional parameters (22–33) whose definitions intuitively follow from above-mentioned descriptions, but they are not mathematically defined here.

Total Probing (or Penetration) Duration (TPD). Sum of probing durations for all insects in a cohort. This parameter estimates reliability of the behavioral record when expressed as percentage of total access time.

$$\mathrm{TPD} = \sum_{i=1}^{n}\sum_{j=1}^{p}\sum_{k=1}^{w}\sum_{l=1}^{e} D_{hijkl} \qquad [2]$$

Total Number of Probes (TNP). Total number of all probes made by all insects in a cohort.

$$\mathrm{TNP} = \sum_{i=1}^{n}\sum_{j=1}^{p} P_{hij} \qquad [3]$$

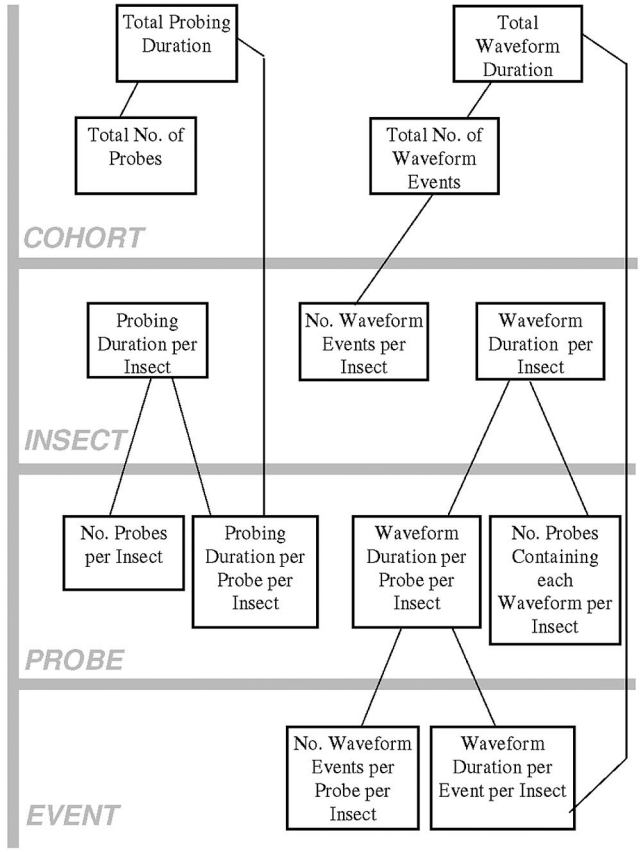

Fig. 1. Hierarchy of heuristic relationships among waveform parameters, at four levels: waveform event, probe, insect, and cohort (Serrano 1997). Two parameters at one level, considered together, are major components of the parameter above them, connected by solid lines. A cohort is the group of insects EPG monitored for one experimental treatment.

Number of Probes by Insect (NPi). Total number of probes made by each insect in a cohort.

$$\mathrm{NPi} = \sum_{j=1}^{p} P_{hij} \qquad [4]$$

When averaged over *N*, this becomes

Number of Probes per Insect (NPI).

$$\mathrm{NPI} = \left(\sum_{j=1}^{p} \mathrm{NPi} \right) \div N \qquad [5]$$

Number of Waveform Events by Insect (NWEi). Number of events of the same waveform performed by an individual insect across all its probes.

$$\mathrm{NWEi} = \sum_{j=1}^{p} \sum_{k=1}^{e} E_{ijkl} \qquad [6]$$

Also defined as the number of events of the k^{th} waveform for the i^{th} insect within the h^{th} cohort.

When this parameter is averaged over *N*, this becomes

Number of Waveform Events per Insect (NWEI). The average number of events of the same waveform, per insect.

$$\mathrm{NWEI} = \left(\sum_{j=1}^{p} \mathrm{NWEi} \right) \div N \qquad [7]$$

Number of Waveform Events by Probe (NWEp). Number of events of a particular waveform within an individual probe.

$$\mathrm{NWEp} = \sum_{l=1}^{e} E_{hijkl} \qquad [8]$$

Also defined as the number of events in the k^{th} waveform in the j^{th} probe of the i^{th} insect in the h^{th} cohort.

Number of Waveform Events per Probe (by Insect) (NWPi). Average number of events of a particular waveform made per probe, for each individual insect in a cohort; calculated as quantity in equation 8 divided by number of probes wherein that waveform was performed (P_{W}).

Table 1. Nonsequential parameters for EPG data mathematically defined in equations 2–21, including abbreviations

Variable name	Abbreviation[a]	Parameter (equation)
Total Probing Duration	TPD	2
Total Number of Probes	TNP	3
Number of Probes by Insect	NPi	4
Number of Probes per Insect	NPI	5
Number of Waveform Events by Insect	NWEi	6
Number of Waveform Events per Insect	NWEI	7[b]
Number of Waveform Events by Probe	NWEp	8
Number of Waveform Events per Probe (by Insect)	NWEPi	9[b]
Number of Waveform Events per Probe (per Insect)	NWEPI	10[b]
Probing Duration by Insect	PDi	11
Probing Duration per Insect	PDI	12
Probing Duration per Probe by Insect	PDPi	13
Probing Duration per Probe per Insect	PDPI	14
Waveform Duration per Event (by Insect)	WDEi	15
Waveform Duration per Event (per Insect)	WDEI	16[b]
Waveform Duration by Probe	WDp	17
Waveform Duration per Probe (by Insect)	WDPi	18
Waveform Duration per Probe (per Insect)	WDPI	19[b]
Waveform Duration by Insect	WDi	20
Waveform Duration per Insect	WDI	21[b]

Parameters originally from Serrano (1997). D, duration; E, event; I, per insect; i, by insect; N, number; W, waveform; P, probe or probing; and T, total.

[a] Also used in equations; lowercase letters designate "by" parameters (see text); uppercase letters designate "per" parameters (see text).

[b] Calculated by dividing by the number of probes and insects that performed that type of waveform.

$$\text{NWEPi} = \left(\sum_{j=1}^{p} \text{NWEp}\right) \div \left(\sum_{i=1}^{n} W_{hijk}\right) \qquad [9]$$

Also defined as the average number of events of the k^{th} waveform in the j^{th} probe of the i^{th} insect in the h^{th} cohort.

Table 2. Nonsequential parameters for EPG data derived from mathematical definitions in Table 1, including abbreviations

Variable Name	Abbreviation[a]	Parameter
Total Waveform Duration	TWD	22
Total Number of Insects by Waveform	TNIw	23
Total Number of Probes by Waveform	TNPw	24
Total Number of Probing Events	TNPE	25
Total Number of Waveform Events	TNWE	26
Number of Probes Containing Each Waveform by Waveform by Insect	NPWi	27
Number of Probing Events by Insect	NPEi	28
Number of Waveform Events per Probe	NWEP	29
Probing Duration per Event	PDE	30
Probing Duration per Event (by Insect)	PDEi	31
Probing Duration per Probe	PDP	32
Waveform Duration per Event	WDE	33
Number of Probes Containing Each Waveform by Waveform per Insect	NPWI	34

Parameters originally from Cline and Backus (2000). D, duration; E, event; I, per insect; i, by insect; N, number; W, waveform; P, probe or probing; and T, total.

Number of Waveform Events per Probe (per Insect) (NWEPI)

$$\text{NWEPI} = \left(\sum_{j=1}^{n} \text{NWEPi}\right) \div N \qquad [10]$$

Probing (or Penetration) Duration by Insect (PDi). Sum of durations of all probes (i.e., in practice, the sum of all waveform events, regardless of type) made by an individual insect.

$$\text{PDi} = \sum_{j=1}^{p} \sum_{k=1}^{w} \sum_{l=1}^{e} D_{hijkl} \qquad [11]$$

Also defined as total duration of probing of the i^{th} insect within the h^{th} cohort.

When divided by N, this becomes

Probing (or Penetration) Duration per Insect (PDI). Amount of time an average insect had stylets inserted.

$$\text{PDI} = \left(\sum_{i=1}^{n} \text{PDi}\right) \div N \qquad [12]$$

Probing (or Penetration) Duration per Probe (by Insect) (PDPi). Average duration per probe made by an individual insect.

$$\text{PDPi} = \left(\sum_{j=1}^{p}\right) \div D_{hij} \qquad [13]$$

Also defined as the average duration per probe for the i^{th} insect within the h^{th} cohort.

When averaged over N, this becomes

Probing (or Penetration) Duration per Probe (per Insect) (PDPI). Average per insect of quantity in equation 13.

$$\text{PDPI} = \left(\sum_{j=1}^{n} \text{PDPi}\right) \div N \qquad [14]$$

Waveform Duration per Event by Insect (WDEi). Duration of the average event of a specific waveform type across all probes of each individual insect. Thus, waveform duration is a measure of a single waveform type, in this case, per event.

$$\text{WDEi} = \left(\sum_{j=1}^{p} \sum_{l=1}^{e} D_{hijkl}\right) \div \left(\sum_{j=1}^{p} \sum_{l=1}^{e} E_{hijkl}\right) \qquad [15]$$

Also defined as the average duration of events of the k^{th} waveform of the i^{th} insect in the h^{th} cohort.

Because this mean is calculated for each individual insect (i), it can be used for statistical analysis, and could be averaged per probe (e.g., equations 17 and 18) or per insect (e.g., equations 16, 19, 20, and 21).

When averaged over *N*, this becomes

Waveform Duration per Event (per Insect) (WDEI).

$$\text{WDEI} = \left(\sum_{j=1}^{n} \text{WDEi} \right) \div N \qquad [16]$$

Waveform Duration by Probe (WDp). Because more than one event of the same waveform can take place during a probe, a parameter that sums durations of all events of the same waveform within a probe is needed:

$$\text{WDp} = \sum_{l=1}^{e} D_{hijkl} \qquad [17]$$

Also defined as the sum of the durations of the l^{th} event of the k^{th} waveform in the j^{th} probe of the i_{th} insect in the h^{th} cohort.

Waveform Duration per Probe (by Insect) (WDPi). Average duration of a particular waveform (i.e., all events summed) per the probes wherein it occurred.

$$\text{WDPi} = \left(\sum_{j=1}^{p} \sum_{l=1}^{e} D_{hijkl} \right) \div \left(\sum_{j=1}^{p} W_{hijk} \right) \qquad [18]$$

Also defined the average duration per probe of the k^{th} waveform of the i^{th} insect in the h^{th} cohort.

When averaged over *N*, this becomes

Waveform Duration per Probe (per Insect) (WDPI).

$$\text{WDPI} = \left(\sum_{i=1}^{n} \text{WDPi} \right) \div N \qquad [19]$$

Waveform Duration by Insect (WDi). Sum of durations of all events of one waveform type (w) made by each individual insect that produced that waveform.

$$\text{WDi} = \sum_{j=1}^{p} \sum_{l=1}^{e} D_{hijkl} \qquad [20]$$

Waveform Duration per Insect (WDI). Average of previous quantity over *N*.

$$\text{WDI} = \left(\sum_{j=1}^{n} \text{WDi} \right) \div N \qquad [21]$$

Organizational Levels for Analysis. Mathematical relationships among waveform variables can be organized into a hierarchy (Fig. 1), which can be a powerful heuristic tool for understanding overall probing behavior. Event level data (bottom of the chart) are the basic units of EPG data. This level is where most researchers begin (and sometimes end) their analyses; however, additional insight can be gained from analysis of higher levels. The probe level combines event level data to produce characteristic information from stylet insertion until removal. These data differ from the event level because they provide sequence information, transition information, or both when more than one waveform event occurs during a probe. The insect level provides information about behaviors of each individual insect as well as those of a single average insect in the cohort, which may approach the population mean. Cohort level data also are useful for understanding the behavioral repertoire of a population.

Parameters that describe probing behavior at one level often are mathematically and heuristically related to one or more parameters at a lower or higher levels. For example, WDI (at the insect level) can be thought of as a combination of WDPI and NPWI. Whereas each parameter may be thought of as residing at one level (e.g., the latter two reside at probe level in Fig. 1), relationships among parameters at different levels can be discussed from the point of view of either level (e.g., insect or probe). Our SAS program can quickly generate values for all of these parameters from an EPG data set as well as a transitional matrix for conditional probabilities (SAS program available upon request from EAB). Herein, we use 11 of the 32 parameters listed in Tables 1 and 2, for descriptive purposes, not hypothesis testing. Some of these parameters also have been used for hypothesis testing (Serrano 1997, Serrano et al. 2000, Almeida and Backus (2004).

Analysis of *L. hesperus* Data. EPG data were subjected to two SAS procedures. The first procedure provided descriptive statistics for feeding behaviors at all levels. Parameters that were biologically relevant to feeding behaviors of *L. hesperus* were compared among waveform types by using analysis of variance (ANOVA), and subsequent pairwise comparisons were made using Fisher protected least significant difference (LSD) (PROC GLM) test (SAS Institute 1992). Data were log- and square root-transformed before ANOVA to reduce variability and improve homogeneity. The second procedure was sequential analysis (via transitional matrix) of nonsequential parameters (i.e., not using sequential parameters). From these data, conditional probabilities were derived that determined probability of occurrence for all behaviors. Video data were subjected to the same sequential analysis as the waveform data.

Review of *L. hesperus* Waveform Definitions (Cline and Backus 2002)

Waveform A. Actually, a composite of several subtypes, A is a highly stereotypical, short-duration (1.5–7-s) "test" probe with both sensory and preparatory functions (Cline and Backus 2002). Subtype A1 is a high-amplitude insertion spike, representing deep stylet drilling and probable watery salivation. Subtype A2 is a lower amplitude signal similar to C1 (see below) ingestion, implying brief fluid uptake for gustation. Subtype A3 is similar to A1 but slightly lower amplitude. This A3 "pull-out spike" represents stylet removal and further saliva secretion.

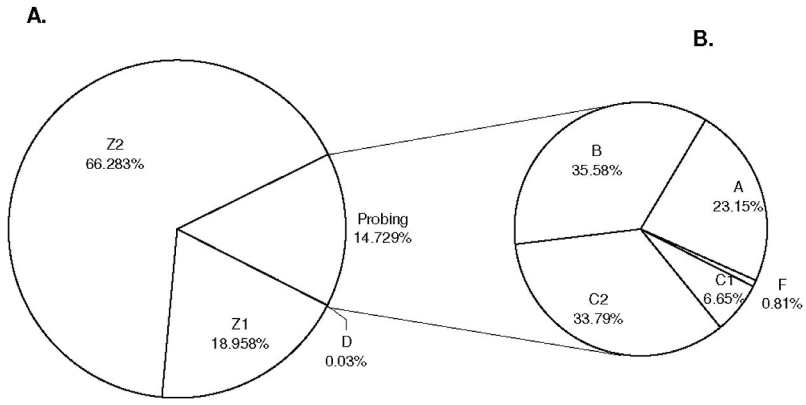

Fig. 2. (A) Total percentage of time spent in moving (Z1), standing (Z2), or probing. (B) Percentage of TPD spent in each waveform: flat-line ingesion (C1), wavy-line ingestion (C2), drilling/laceration/salivation (B), test probe (A), excretion (D), and extended labial dabbing (F).

Waveform B. This waveform is characterized by moderate-amplitude, irregular spikes with occasional high peaks or low valleys. Laceration, or stylet drilling via alternate protraction and retraction, is diagnostic of waveform B. The labium makes multiple single bends and, in the deepest penetrations, can make a second bend between the terminal and penultimate segments (Cline and Backus 2002). Copious watery salivation (maceration) also is typical, and sometimes salivation occurs while stylets are motionless. Waveform B is performed at variable depths, both on the plant surface and penetrating deeply into underlying tissues.

Waveform C. Previously correlated with particle movement toward the stylets in artificial diet (Cline and Backus 2002), C represents ingestion and primary food uptake. In contrast to B, labial angles are static, because the stylets are held at a fixed depth, suggesting a preferred ingestion depth (Cline and Backus 2002). Waveform C has two subtypes based on appearance. Subtype C1 is a moderate-amplitude, steady waveform with very small, regular spikelets. C2 is similar, but with frequent, slightly higher, wave-like peaks, probably representing spurts of watery saliva during otherwise uninterrupted ingestion (Cline and Backus 2002).

Waveform F. Rarely observed, F is similar to B but higher amplitude. It represents rapid protractions and retractions of stylets into and out of the plant tissues, at very shallow depths.

Waveform Z. Nonprobing waveform, Z, is a very low-amplitude signal, synonymous with baseline. It has two distinct subtypes in *L. hesperus* recordings. Subtype Z1 is highly irregular, spiky to wavy, and correlated with nonprobing movements made by the insect on the plant, such as walking, antennation, labial dabbing, and grooming (see Videomicrography Data below). Subtype Z2 is a flat baseline, with very little change in voltage. It is recorded during motionless standing (Cline and Backus 2002). Detecting body postures (i.e., standing motionless versus moving) is only possible, however, during nonprobing. During probing, the higher amplitude waveforms mask the baseline waveforms.

Table 3. Cohort and event waveform values (mean ± SE)

Variable	Abbreviation (para. no.)	Main probing waveforms			
		A	B	C1	C2
Cohort level					
Total waveform duration (s)	TWD (21)	4045.7	6216.1	1162.3	5903.9
Percentage of TPD		23.2	35.6	6.7	33.8
Event level					
Total no. waveform events	TNWE (25)	763	226	15	29
Waveform duration/event (/insect) (s)	WDEI (15)	5.9 ± 0.1c	24.6 ± 0.2bc	79.8 ± 1.0b	220.1 ± 0.8a
		Nonprobing or less significant probing waveforms			
		D	F	Z1	Z2
Cohort level					
Total waveform duration (s)	TWD (21)	4.67	141.6	22,444.1	78,475.6
Percentage of total nonprobing duration		0.005	1.0	22.2	77.7
Event level					
Total no. waveform events	TNWE (25)	2	1	16	16
Waveform duration/event (/insect) (s)	WDEI (15)	2.3 ± 0.2c	35.4 ± 0bc	17.4 ± 0.1c	186.9 ± 0.7a

Table 4. Probe and insect level waveform values (mean ± SE)

Variable	Abbreviation (para. no.)	Main probing waveforms			
		A	B	C1	C2
Probe level					
Total no. probes by waveform	TNPw (23)	761	196	12	29
Insect level					
Total no. insects by waveform	TNIw (22)	16	16	8	15
No. waveform events/insect	NWEI (6)	47.69 ± 16.0ab	14.1 ± 2.1bc	1.9 ± 0.64c	1.9 ± 0.3c
Waveform duration/insect	WDI (20)	252.86 ± 75.7cd	388.51 ± 87.5c	145.29 ± 50.5cd	393.59 ± 69.9c
		Nonprobing or less significant probing waveforms			
		D	F	Z1	Z2
Probe level					
Total no. probes by waveform	TNPw (23)	2	2	N/A	N/A
Insect level					
Total no. insects by waveform	TNIw (22)	2	1	16	16
No. waveform events/insect	NWEI (6)	1.00 ± 0c	4.00 ± 0c	88.63 ± 18.4a	32.2 ± 2.9ab
Waveform duration/insect	WDI (20)	2.34 ± 0.1d	141.57 ± 0cd	1402.7 ± 225.1b	4904.7 ± 343.3a

Means followed within a row by a different letter differ significantly ($P < 0.05$), by using protected least significance difference test. N/A, not applicable.

Results

Nonsequential Analysis

Cohort Level. From a total access time of 118,394 s, the 16 insects spent 17,474 s (14.73%) in stylet penetration, i.e., TPD. The remaining time was spent in nonprobing activities such as moving (waveform Z1) or resting (waveform Z2) (Fig. 2). The percentage of TPD represented by each of the four main probing waveforms (each total waveform duration; TWD) was similar for A, B and C2 (Table 3), with waveform C1 performed much less. The TNP was 958, with the PDP being 18.2 ± 1.87s (mean ± SE). Thus, compared with most Auchenorrhyncha and Sternorrhyncha, nymphal *L. hesperus* probing was more mechanically active and broken into numerous, short probes. To understand this behavior more completely, however, we subdivided this cohort-level analysis into the smallest possible unit, i.e., waveform events and then rebuilt back to cohort level via probe and insect levels.

Waveform Event Level. The total number of probing and nonprobing events for all nymphs was 2,972. Of these events, >65% consisted of nonprobing waveforms (i.e., D, Z1, and Z2), which further demonstrated the disproportionate amount of nonprobing behaviors. The most frequent waveform exhibited was Z1, or movement.

ANOVA found significant differences among waveforms ($P < 0.0001$) for WDEI ($F = 32.09$; df = 7, 82). Waveform A, test probe, had the shortest WDEI, 5.3 s per event. However, waveform A had the highest frequency of occurrence (TNWE), 71.4% of all probing events (i.e., uninterrupted events) were waveform A (Table 3). This explains why TWD for waveform A was 23.2% of TPD (Table 3).

Waveform B, "drilling/laceration/salivation," constituted only 23.2% of all probing events, yet had the highest percentage of TPD at 35.6% (Table 3). B also had a high TNWE with a WDEI intermediate between A and C1 (see below). Thus, together A and B comprised >94% of probing events and ≈60% of TPD, although not directly involved with ingestion (fluid uptake past the cibarium) (Cline and Backus 2002).

Waveforms C1 and C2 together represented >40% of TPD; however, these two ingestion waveforms differed in WDEI (Table 3). Mean durations of C2 events were significantly longer, and A events were significantly shorter, than those of both other waveforms. Both C1 and B were intermediate and not significantly different from each other, and B was not significantly different from A (Table 3).

Probe Level. Probe level data were almost identical to event level data because most probes contained only a single waveform event, waveform A. Of the total 958 probes made by the cohort, 761 (79%) were test probes (TNPw; Table 4). NPI was 45.4 ± 2.02. The remaining probes consisted solely of laceration/salivation (B) (16.3%), or B combined with ingestion (C1 and/or C2) (4.2%). Only 12 probes (1.4%) contained C1, and 29 probes (2.8%) contained C2. All ingestion-containing probes began with B and were either terminated directly after ingestion or ended with B. Thus, A always occurred alone in a probe, whereas B occurred either singly or with C1 or C2 in a laceration/salivation-ingestion pattern.

Insect Level. As typical for hemipteran probing, there was high variation among individual insects in the cohort (Fig. 3) for probing and waveform durations by insect (PDi and WDi, respectively, as stacked bars) as well as inferred nonprobing durations (blank areas above bars in Fig. 3). Three of the four major probing waveforms were performed by most insects (A by 16 insects, B by 15, C2 by 15); C1 was performed by only eight insects. Variation in duration of individual behaviors was especially high with C2 (30.7–682.4 s) and Z2 (<30 to >700 s) (Fig. 3). NPi was highly variable, ranging from 5 to 272. Therefore, most nymphs performed numerous, short-duration, laceration events as opposed to fewer, longer duration, in-

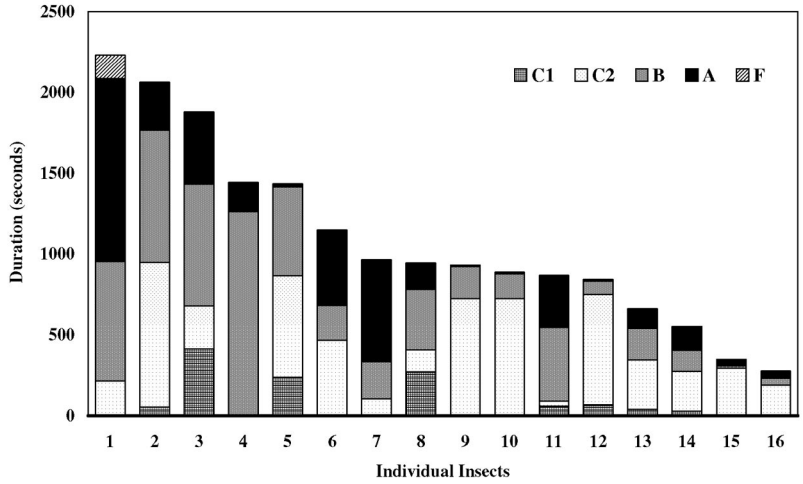

Fig. 3. Individual variability in probing and waveform durations by each insect. Top of each bar corresponds to probing duration for each insect (i.e., PDi). Each bar contains total waveform durations for each insect (i.e., WDi) for behaviors expressed in legend.

gestion-containing events. However, not all insects followed this trend; one insect performed a few long-duration events (one C1, one C2, three B, and two A).

Trends also are revealed by an average insect. WDI is a product of NWEI and WDEI. Therefore, WDI is an indicator of time an average insect spends in a behavior. WDI was significantly different between waveforms ($F = 61.50$; df = 7, 82) due to differences between C1 and A durations per insect; C2, B, and A were not significantly different from one another (Table 4). The number of C1 and C2 events per insect (NWEI) was significantly lower ($P = 0.0001$ for both waveforms) than number of A events, with B events intermediate (Table 4). Combining NWEI with WDEI, the durations and frequencies of A and C2 events were opposite, but they balanced one another, resulting in overall durations per insect (WDI) not significantly different.

Summary. These descriptive statistics revealed three major types of stereotypical behaviors: 1) test probes with very short, frequent, waveform A events performed in isolation within probes; 2) laceration/drilling probes with intermediate durations and frequencies of waveform B, without associated ingestion; and 3) infrequent laceration/salivation-ingestion probes containing B but also long events of waveforms C1 and, especially, C2. Thus, in terms of duration per insect, laceration/salivation-ingestion probes were the primary probing behavior, with test and laceration/salivation probes occurring secondarily.

Conditional Probability Analysis

EPG Data. To test for the stereotype of behavior described above, we derived conditional probabilities (i.e., probability that, given the occurrence of a certain waveform, it will be followed by any other waveform) for event-level variables of probing and nonprobing waveforms (Fig. 4). The total number of behavioral transitions observed was 2,956. Of those events, 35.1% represented probing, mostly stylets moving and salivation (25.8% test probing [A] and 7.5% laceration/salivation [B]) (Table 5). For the nonprobing behaviors, 47.7% of the total transitions involved moving (Z1); 17.1% involved standing (Z2) (Table 5).

Although nearly two thirds of an average insect's time was spent standing (Z2), stylet penetration was rarely initiated from this posture (Fig. 4). Instead, 97.6% of the time, an insect moved (Z1) to a new position before initiating a probe. From Z1, a test probe (waveform A) was most often performed. Waveform A was rarely (<0.01%) associated with another waveform. After completion of A, the insect returned to nonprobing, usually Z1 (96.6%) (Fig. 4).

Subtype Z1 also led to either a B or C2 behavior, although more often to B than directly to C2. Interestingly, once B was performed, the insect frequently returned directly to nonprobing behavior. Waveform B was sometimes followed by ingestion. Ingestion behaviors frequently returned to B. This revealed a B → C → B sequence (i.e., laceration/salivation, ingestion, laceration/salivation), which was found in 15 of 16 insects. All C-type ingestion was correlated with at least one B event 100% of the time. Most probes terminated into Z1 (moving) rather than Z2 (standing). Thus, most probes were preceded and followed by some movement (most often antennation, walking, or labial dabbing, in that order; see below), with standing done away from the probing site.

Videomicrography Data. Computerized time-synchronization technology of video and EPG signals was not available when this research was performed. During analysis, it became apparent that EPG and video signals were temporally and variably uncoordinated, usually by 1–6 s but rarely as long as 15 s. Therefore, we were unable to statistically correlate EPG and video data directly. However, our visual correlation between video and EPG recordings contributed to

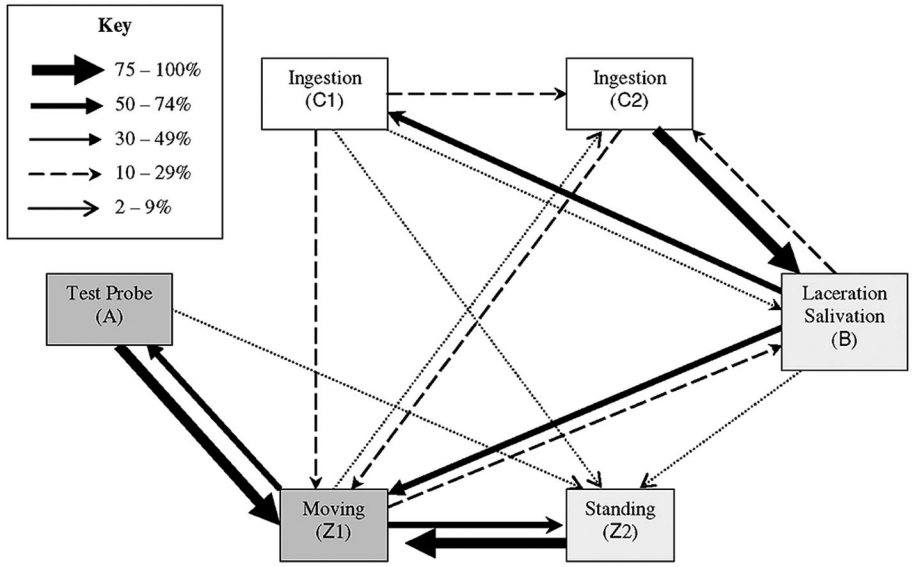

Fig. 4. Kinematic diagram of EPG data, showing probing and nonprobing behaviors. A, test probe; B, drilling/laceration/salivation; C1, flat-line ingestion; and C2 wavy-line ingestion. Arrows connect two behaviors whose probability of occurrence is indicated by line weights in legend. Ends of arrows denote preceding behaviors; arrowheads denote following behaviors. Any behavioral transition whose conditional probability was <2% is not shown. No boxes are present for behaviors with <20 transitions (Table 5). This explains why waveforms D and F are not displayed. Dark gray boxes represent >750 transitions for each behavior (see Table 5); light gray represents 100–749 transitions; white boxes represent <100 transitions.

further understanding of waveform meanings and behaviors. For example, stylet movements were readily observed and visually correlated with A and B waveforms, due to rapid changes in labial angles (Cline and Backus 2002).

Video observations confirmed EPG findings that only a minority of the insect's access time was spent probing. Video results added to the EPG findings by showing that 18 body posture/movement behaviors occurred during Z1 and Z2. These behaviors were given Ω designations, which are briefly defined here (Table 5) (derived from Cline and Backus 2002). Visual correlations showed that EPG waveform Z1 (movement) corresponded to all omega behaviors

Table 5. Total number of behavioral transitions performed preceding or following a behavior (probing and nonprobing) observed via EPG and videomicrography

EPG behavior	Preceding[a]	Following[b]	Video behavior	Preceding[a]	Following[b]
EPG probing behavior			Video probing behavior		
Test probing (A)	763	763	Stylets in fixed position (γ)	1,374	1,365
Laceration/salivation (B)	226	226	Stylet drilling/laceration (β)	363	406
Flat ingestion (C1)	15	15	Test probe (α)	53	51
Wavy-line ingestion (C2)	29	29	Excretory droplet (δ)	6	5
Excretory droplet deposit (D)	2	2	Salivary bubble (ε)	3	4
Repetitive stylet insertion (F)	4	4			
EPG nonprobing behaviors			Video nonprobing behavior		
Moving (Z1)	1,410	1,414	Antennation (Ω4)	1,020	946
Standing (Z2)	507	503	Standing (Ω2)	899	901
			Walking (Ω1)	796	806
Total transitions	2,956	2,956	Labial dabbing (Ω11)	188	216
			Insect out of field (Ω15)	94	95
			Foreleg tarsal grooming (Ω6)	58	59
			Midleg/hindleg grooming (Ω8)	56	50
			Foreleg/midleg grooming (Ω7)	44	45
			Antennal grooming (Ω5)	43	45
			Reorientation (Ω3)	20	25
			Labial grooming w/ foreleg (Ω10)	17	15
			Labial dragging (Ω14)	8	6
			Hindleg tarsal grooming (Ω9)		
			Total transitions	6,122	6,122

Letters and numbers in parentheses correspond to behavioral codes described in Cline and Backus (2002).
[a] Number of transitions in which behavior preceded another observed behavior.
[b] Number of transitions in which behavior followed another observed behavior.

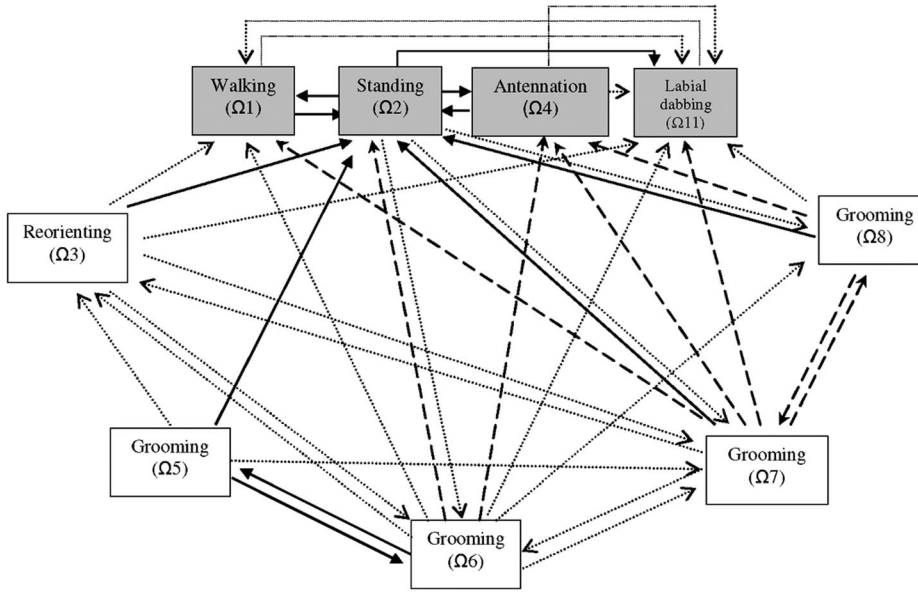

Fig. 5. Kinematic diagram of videotaped nonprobing behaviors. Line weights and box formats as described for Fig. 4.

(Table 5) other than standing, i.e., walking (Ω1), reorienting the body (Ω3), antennation (Ω4), some type of grooming (Ω's 5, 6, 7, 8, 9, and 10) or labial dabbing (Ω11). Subtype Z2 corresponded solely to standing motionless (Ω2) (Table 5). Designations α, β, λ, δ, and ζ represent probing behaviors visible as head, stylet, or labial movements. Behavior α was assigned to test probing and was associated with waveform A. Although test probes were recognizable with EPG, they were often difficult to recognize with video, due to their highly stereotypical appearance, brevity, and rapid stylet movements. Likewise, β was assigned during active, vertical stylet movement, often associated with waveform B. Behavior γ was assigned when stylets were motionless (i.e., labial angles unchanging) within the plant. This often occurred during ingestion, although C1 and C2 could not be differentiated with video analysis (Table 5).

The total number of behavioral transitions videotaped was 6,122. Of those, 29.2% represented probing (0.9% test probing [α], 5.9% stylet drilling [β], 22.4% stylets in fixed position [γ]). For the nonprobing behaviors, 62% of the total transitions involved four primary behaviors (13–18% each), which both preceded and followed most probing. These were (in decreasing order of performance): antennation (Ω4), standing (Ω2), walking (Ω1), and labial dabbing (Ω11). Discounting 'insect out of field of view' (3.1%), all but one of the other nonprobing behaviors (mostly grooming) each represented <1% of the total transitions; foreleg tarsal grooming (Ω6) represented 1.5% (Table 5).

Two kinematic diagrams of the conditional probabilities show the relationships among these four primary transitional nonprobing behaviors and other nonprobing behaviors (Fig. 5) or probing behaviors (Fig. 6). Standing motionless is the central behavior, after which the insect was most highly (and equally) likely to begin walking, antennating, or labial dabbing (Fig. 5). Less likely but consistently, the insect could perform any of several different grooming behaviors. Although walking and antennation could lead back to standing, labial dabbing did not (Fig. 5). Instead, dabbing always led to probing, especially stylet drilling and stylets motionless (Fig. 6). Secondarily, drilling and stylets motionless were preceded by antennation. Walking occasionally led to probing. However, in contrast to the EPG data, test probing rarely followed any of the four key nonprobing behaviors but mostly followed drilling. We suspect this was an artifact of the difficulty of recognizing test probes by video observations and that more often they were classified as stylet drilling (Fig. 6).

Interestingly, the most common probing behaviors, drilling and stylets motionless, did not directly precede or follow one another. Instead, primarily antennation occurred briefly between drilling and stylets motionless (Fig. 6). Labial dabbing occurred if the insect terminated the probe. This finding was unique to video observation, because EPG could not capture other body movements during probing.

Thus, if Figs. 5 and 6 are overlaid (Fig. 5 on the bottom, Fig. 6 on the top, with the four primary behaviors in the center), a behavioral pattern emerges. From a standing position, a *L. hesperus* nymph most likely proceeded to alternate antennation and labial dabbing, sensorially testing the plant surface (i.e., host plant acceptability). After a last event of labial dabbing, the insect penetrated the plant with its stylets and then alternated drilling with stylets motionless, performing antennation as it transitioned from one to

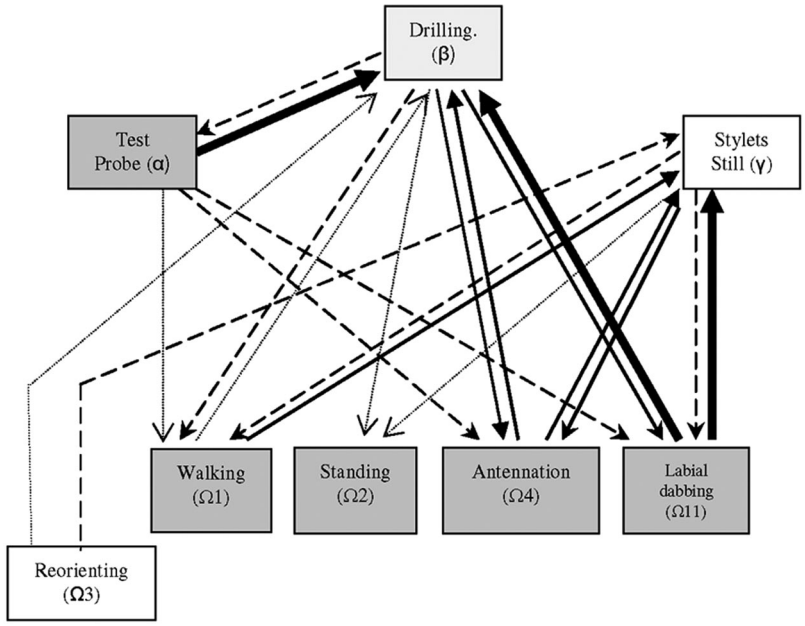

Fig. 6. Kinematic diagram of videotaped probing behaviors. Line weights and box formats as described for Fig. 4.

the other. Test probing also was performed but it was not documented via video as well as EPG, because shallow, brief probes are difficult to recognize visually. After termination of probing, the insect usually walked away rapidly from the probe site for a second or two before beginning another probe. Alternatively, after walking, it might have stood longer, or groomed a variety of appendages. Therefore, using the video data, we can expand the behavioral sequence as follows: 1) walking, 2) sensory assessment of a possible probing site, 3) probing, 4) more walking, 5) standing/ grooming. In conclusion, video observation only partially confirmed the behavioral sequence of B → C → B observed with EPG, and it also provided additional information not documented by EPG.

Discussion

New Statistical Methods and Parameters. We provide here the first complete system of organization and terminology for nonsequential EPG parameters. These parameters, demonstrated via *L. hesperus* descriptive statistics, provide a deeper understanding of the meanings and relationships among EPG waveforms than many previously published parameters. The parameters and organization can be applied to other hemipteran systems, leading to greater utility of EPG in other research. In addition, they allow powerful statistical tools for testing among various treatments in an experiment. For example, principal component analysis of a selection of our parameters led to the first successful stylet penetration index (SPI), a resistance index for comparison among resistant and susceptible crop varieties that relies exclusively on EPG data (Serrano et al. 2000). Widespread adoption and use of these terms will allow standardization in EPG research.

Implications of *L. hesperus* Findings for Plant Damage Mechanisms. Our EPG and video data reveal for the first time the stylet penetration behaviors of nymphal *L. hesperus* that cause cotton square damage, and the mechanism involved. Our *L. hesperus* nymphs performed dozens of probes per hour on a cotton square, actively moving among different probing sites across the square. Some individual insects performed >100 test probes during a 2-h access time; but, on average, they performed 45 test probes. Nearly 80% of an insect's probes (NPI) were very short (≈6 s) test probes (WDE); 16% of probes were involved in longer durations (≈25 s) of exclusively laceration/ drilling/ salivation; <5% of probes involved ingestion. Each ingestion probe lasted on average 2.5 min for C1 and 6.5 min for C2. Thus, two thirds of TPD was spent in laceration/salivation and one third in ingestion. Even more significantly, each insect spent only 15% of its access time probing; the remaining access time was spent primarily standing motionless in place, grooming, or in sensory exploration via antennation and labial dabbing.

The data support that *L. hesperus* nymphs actively spread out numerous, minute injections of their watery saliva into all parts of the developing square. This biochemically active, macerating saliva has potent cell wall-degrading enzymes such as polygalacturonase (PG), as originally demonstrated by Strong and Kruitwagen (1969). Subsequently, Strong (1970) proposed that the major mechanism of *Lygus* feeding damage is tissue maceration by this salivary PG. Recent work (Shackel et al. 2005) experimentally supported this conclusion, by showing that tissue damage mimicking

Lygus-induced necrosis can be induced artificially in the absence of an insect's laceration behavior by injecting ≈300 nl of *L. hesperus* saliva into an alfalfa floret. In light of this finding, we suggest that the drilling/laceration behavior of *L. hesperus* nymphs functions primarily for deep placement and spread of the saliva. The extensive laceration by the stylets may, secondarily, potentiate salivary maceration by mechanically rupturing cell walls that can then be more quickly degraded by PG and other salivary enzymes

After broadcast injection of saliva within the square, the insect then stands and waits for solubilization of the square's cell contents, after which it quickly ingests the slurry. With only one third of its probing time spent in ingestion, *L. hesperus* nymphs may not be able to ingest all of the saliva/cell content slurry they create. This may be especially true of early instars. If some saliva is left in the plant, it may continue to negatively affect normal plant physiology long after insect departure. The subsequent plant reactions could partly explain the highly damaging nature of *Lygus* probing compared with other cell rupture feeders. The damage documented by Leigh et al. (1988) of 23,400 cotton squares per acre destroyed by a single *Lygus* bug (a damage rate far in excess of comparable feeding by, e.g., *Empoasca* leafhoppers) can thus be explained by biochemically active saliva interacting (both short-term and long-term) with mechanically ruptured germ cells, long after feeding terminated. Long-term effects of *Lygus* feeding could therefore be a combination of saliva-initiated plant responses and wound responses.

Similar probing behaviors by *Empoasca* leafhoppers initiate a cascade of plant responses that cause the plant disease called hopperburn. In hopperburn, the plant response is summarized as a "saliva-enhanced wound response" (Backus et al. 2005), because salivary effects are less drastic than those of *Lygus*, and mechanical laceration of vascular tissues results in severe wound responses in the cambium tissue. In contrast, for *Lygus*, the plant responses could be summarized as "mechanical cell rupture-enhanced maceration."

Implications of *L. hesperus* Findings for Hemipteran Feeding Strategies. Miles (1972) identifies two overarching feeding strategies used by phytophagous hemipterans, i.e., stylet (more accurately, salivary) sheath feeding and lacerate-and-flush feeding. The latter usess vigorous stylet movements (to lacerate) and copious watery salivation in absence of a salivary sheath (to macerate) contents of primarily parenchyma/mesophyll cells (although not exclusively; hopperburning *Empoasca* probe vascular tissues; Backus et al. 2005). After the work of Miles (1972), EPG began to be used to study lacerate-and-flush feeders such as typhlocybine leafhoppers. Backus et al. (2005), in their review of >20 years' work on *Empoasca* spp. leafhoppers, propose a new term, cell rupture feeding, as a more general category that includes lacerate-and-flush as well as other types of feeding. EPG was the crucial research method in that work, and it clarified many previously unanswered questions. It is now understood that cell rupture feeding, in turn, comprises several related stylet penetration tactics (i.e., substrategies, recognizable as stereotypical, sequential combinations of EPG waveforms (Serrano 1997, Serrano et al. 2000). These combinations are lacerate-and-sip, lacerate-and-flush, and lance-and-ingest; all three tactics are used by *Empoasca* spp. leafhoppers.

Miles and Taylor (1994) coined the term macerate-and-flush to denote the probing strategy of *H. clavifer*. This insect inserts its stylets directly into a pocket of parenchyma/mesophyll cells with (according to visual observation) little or no laceration. Rapid injection of saliva and subsequent ingestion of macerate follow. Miles and Taylor (1994) did not document the time between salivation and ingestion events, nor has any EPG or video study of this species been performed. However, on the basis of their observation of *H. clavifer*, Miles and Taylor (1994) suggest that macerate-and-flush is a more appropriate strategy for all mirids (i.e., all Cimicomorpha?), whereas lacerate-and-flush is performed by lygaeids and pentatomids (i.e., all Pentatomomorpha?). No mention of typhlocybine leafhoppers such as *Empoasca* spp. is made.

Thus, lacerate-and-flush and macerate-and-flush are closely related. Moreover, the terms are rather confusing because both tactics perform maceration. The real distinction between the two is in the amount of active stylet movements/wounding and in the degree of biochemical activity of the saliva. Lacerate-and-flush feeders perform very active, lacerating stylet movements. Their saliva, although containing some cell wall-degrading enzymes such as polygalacturonase (Shackel et al. 2005), seems less active and to require some time to solubilize cell contents. In contrast, macerate-and-flush feeders (at least *H. clavifer*) perform almost no stylet laceration, because their saliva is highly enzymatically active. We propose that both macerate-and-flush and lacerate-and-flush are more logically categorized as stylet penetration tactics within cell rupture feeding, not a full strategy as originally proposed by Miles and Taylor (1994).

The current study shows exactly how cell rupture feeding is performed by *L. hesperus*, and it demonstrates how video analysis combined with EPG rigorously revealed the true nature of feeding. Waveforms B of *L. hesperus* and I_c of *Empoasca* spp. are clearly recognizable as laceration/salivation. Wavy-line ingestion such as the C2 waveform of *L. hesperus* or the I_b waveform of *Empoasca* spp. leafhoppers represents "flushing" behavior (Backus et al. 2005), i.e., brief spurts of watery salivation interspersed with ingestion to flush out cell contents (Miles 1972). Thus, B → C2 → B probes of *L. hesperus* are classical lacerate/macerate-and-flush probes. It seems that B → C1 → B probes are lacerate/macerate-and-ingest probes; a tactic not yet identified. Nonetheless, assigning full tactic status should await a more in-depth, comparative analysis of adult as well as nymphal *Lygus* feeding.

We propose, however, that a behavioral continuum probably exists between the extremes represented by *Lygus*/*Empoasca*-style lacerate-and-flush and the *H. clavifer* macerate-and-flush. Clearly, more informative

names for the tactics in the cell rupture strategy are needed. However, it is premature and not in the scope of this article to propose such a reorganization. At present, it is best concluded that *L. hesperus* nymphs on cotton perform cell rupture feeding by using a mixture of laceration and maceration tactics.

In conclusion, this research is the first attempt to quantify and statistically analyze via EPG the stylet probing/penetration behaviors of *Lygus* bugs as well as the external body movements associated with both probing and nonprobing. Behavioral quantification via EPG allows statistical comparisons among host plants or other treatments, as demonstrated with the methods and parameters defined herein. Such quantification aids in development of a resistance index, or SPI (Serrano et al. 2002), which distills the full feeding repertoire of an insect into a single numerical value statistically comparable between plant genotypes. An SPI can be used to screen plant genotypes for resistance to *Lygus* damage, accelerating the development of resistant cultivars.

The current study lays the groundwork for future research on 1) chemistry of *Lygus* bug feeding and damage to host plants, by using EPG-standardized amounts of feeding; 2) comparisons between feeding behaviors of adults and nymphs; 3) feeding of adults and/or nymphs on different host plants, and 4) comparisons with other cimicomorphan heteropterans.

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